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## ATTACHMENT G

# Evidence for the primary role of anagrelide's major metabolite, 3-hydroxy anagrelide in the drug's clinical activity.

*This represents a summary of Shire data on file.*

## Synopsis

Anagrelide is extensively metabolised in man to two major metabolites; 3-hydroxy anagrelide (also known as SPD604, BCH24426 or 3-HA) 6,7-dichloro-3-hydroxy-1,5-dihydro-imidazo[2,1-*b*]quinazolin-2-one and RL603, 2-amino-5, 6-dichloro-3, 4-dihydroquinazoline. Earlier pharmacokinetic studies in volunteers have recently been followed by a study in patients with essential thrombocythemia (ET) or other myleproliferative diseases where a notable difference was observed. Patients had much greater exposure (>2 fold) to 3-HA than volunteers possibly due to a longer half-life. 3-HA was found to be the major circulating component in blood representing ~45% of all drug related products in the plasma. The other metabolite RL603 constituted ~33% of the plasma components in these patients. Anagrelide itself represented ~20% of the plasma constituents. Anagrelide had the shortest half-life of 1.7h, followed by 3-HA with a half-life of 3.9 and finally RL603 with a half-life of 8.7h.

Pharmacological evaluation of anagrelide and its metabolites showed that, 3-HA had a comparable inhibitory effect to the parent drug on megakaryocytopoiesis - and potentially therefore platelet formation - while RL603 was inactive. Anagrelide and 3-HA were also found to be inhibitors of PDEIII although 3-HA was almost forty times more potent than the parent drug while RL603 was again virtually inactive. *In vivo* studies in dogs showed that this PDEIII inhibitory activity translated into the expected cardiovascular effects resulting in lowered blood pressure, increased heart rate and increased force of cardiac contraction. Additionally, PDEIII inhibition in the blood platelets resulted in an anti-aggregatory effect with the metabolite being at least 12-13 times more potent than anagrelide.

These data together with the clinical pharmacokinetics are summarised in the in-text table below:-

| Compound | *% Total plasma exposure in ET patients* | Plasma t½ | Inhibition of megakaryocytopoiesis ($IC_{50}$) | Inhibition of phosphodiesterase III ($IC_{50}$) |
|---|---|---|---|---|
| Anagrelide | ~20% | 1.7h | 27+/- 10 nM | 32nM |
| BCH24426(3HA) | ~45% | 3.9h | 48+/- 13 nM | 0.9nM |
| RL603 | ~33% | 8.7h | Inactive | 40,000nM |

In view of the significantly greater exposure to 3-HA than to the drug and considering their relative pharmacological potencies, it is probable that this metabolite contributes

most of the platelet-lowering activity and almost all of the cardiovascular side effects seen in patients treated with anagrelide. Any anti-aggregatory effects would also most likely be due to 3-HA.

Thus the role of 3-HA would appear central to the activity of anagrelide. This contention is supported by the results of an exploration of possible pharmacokinetic–pharmacodynamic relationships in ET patients. This showed a good correlation between chronic exposure to the *active metabolite* and the magnitude of platelet lowering as well as with heart rate but a weaker correlation for the drug itself with respect to platelet lowering and no correlation at all with increase in heart rate.

Thus it would appear that the most appropriate barometer of anagrelide's effects would be plasma levels of 3-HA rather than anagrelide itself.

## 1. Pharmacokinetics of anagrelide and its metabolites

### *1.1 Disposition in healthy volunteers*

Data from 38 healthy volunteers (age 21-70yrs of age - mean 52) who participated in three separate clinical pharmacokinetic studies on the drug has provided the basis for an overview of pharmacokinetics of anagrelide and its two major metabolites 3-hydroxy anagrelide (otherwise known as BCH24426, SPD604 or 3-HA) 6,7-dichloro-3-hydroxy-1,5-dihydro-imidazo[2,1-*b*]quinazolin-2-one and RL603, 2-amino-5,6-dichloro-3,4-dihydroquinazoline. All subjects ingested a 1mg dose of anagrelide following an overnight fast.

A tabulation of the mean pharmacokinetic parameters is presented in Table 1 while the comparative pharmacokinetic profile of anagrelide and its two metabolites is shown in Figure1.

$T_{max}$ (mean ± relative standard deviation) for anagrelide was 1.3 hours ± 53.8% indicating rapid absorption of the drug. The mean $C_{max}$ value was 4.99 ng/mL ± 74.4% while exposure, in terms of $AUC_{0\text{-}inf}$ was 11.1 ng·h/mL ± 37.6%. Elimination proceeded rapidly in a mono-exponential manner with a mean half-life of 1.5 hours ± 49.8%.

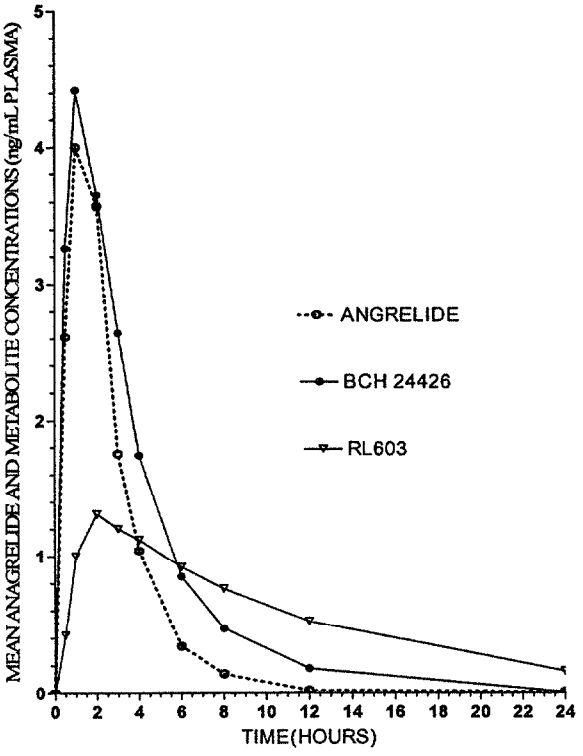
With respect to the kinetics of the active metabolite, 3-HA, the overall mean $C_{max}$ was slightly higher than that for the drug at 5.47ng/ml ± 56.9% and was achieved at a $T_{max}$ of 1.28 hours ± 58.1%. Exposure amounted to 18.0 ng·h/mL ± 35.6 %, some 60% greater than that for the drug itself. The mean half-life of elimination was 2.5 hours ± 28.7%.

For the inactive metabolite, RL603 the mean $T_{max}$ was reached at 2.5 hours ± 58.5% and the corresponding mean $C_{max}$ was 1.36ng/mL ± 34.0%. The mean $AUC_{0\text{-}inf}$ was 16.0ng·h/mL ± 32.3% and mean half-life of elimination was comparatively long at 7.8 hours ± 31.1%.

**Attachment G Table 1: Summary of mean pharmacokinetic parameters of anagrelide and BCH24426 from 38 volunteers given a single 1mg dose of Agrylin**

| Compound | $AUC_{0\text{-}inf}$ ± RSD (%) (ng·h/mL) | $C_{max}$ ± RSD (%) (ng/mL) | $T_{max}$ ± RSD (%) (h) | $t_{1/2}$ ± RSD (%) (h) |
|---|---|---|---|---|
| Anagrelide | 11.1 ± 37.6 | 4.99 ± 74.4 | 1.3 ± 53.8 | 1.5 ± 49.8 |
| BCH24426 (3-HA) | 18.0 ± 35.6 | 5.47 ± 56.9 | 1.28 ± 58.1 | 2.5 ± 28.7 |
| RL603 | 16.0 ± 32.3 | 1.36 ± 34.0 | 2.5 ± 58.5 | 7.8 ± 31.1 |

**Attachment G Figure 1: Mean plasma concentrations of anagrelide, 3-hydroxy anagrelide and RL603 in volunteers**




These data presented graphically in Figure 1, show the somewhat greater exposure to 3-hydroxy anagrelide than to the parent drug. Although the mean $C_{max}$ values did not differ that markedly being 5.47ng ng/ml and 4.99/ml for 3-hydroxy anagrelide (BCH24426) and anagrelide respectively, the ratio of metabolite to drug exposure (AUC) was 1.6 :1.

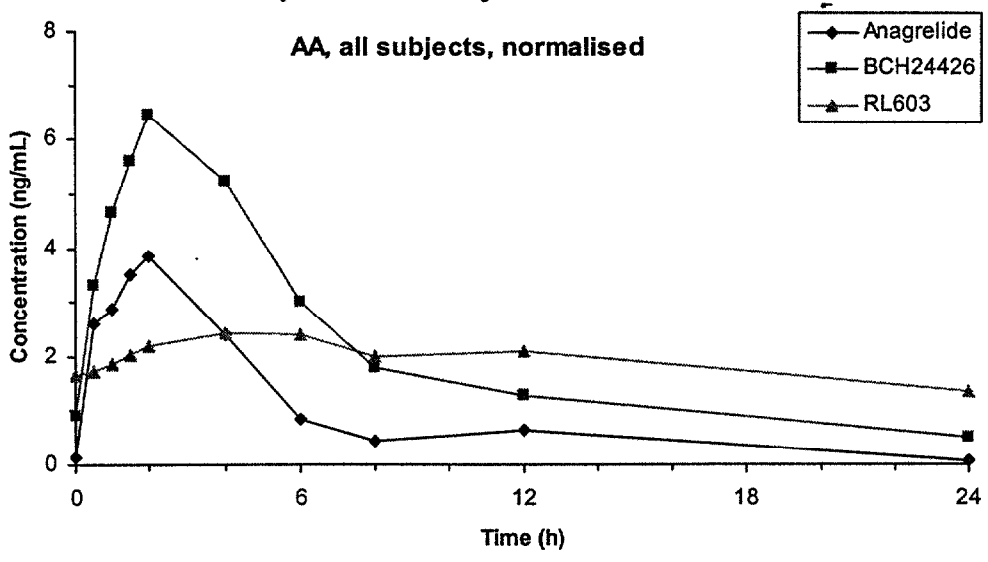
***1.2 Disposition in patients with essential thrombocythemia.***

The pharmacokinetics of anagrelide and its metabolites were recently investigated in patients with essential thrombocythemia (ET). This study involved a comparison of paediatric patient group (<15years of age) with a more representative adult group of patients (16 –86 years of age – mean 63). Patients were being treated with a variety of different dose levels. Consequently the derived pharmacokinetic parameters were normalised to a 1mg dose and body weight to 70kg. Data from the more representative adult patient group are presented in the Table 3 and Figure 2 below.

**Attachment G Table 3: Summary of mean pharmacokinetic parameters of anagrelide and BCH24426 in adult patient group (n=18) at steady state (data normalised to 1mg dose and 70kg body weight)**

| Compound | $AUC_{0\text{-}tau}$ ±RSD (ng·h/mL) | $C_{max}$ ±RSD (ng/mL) | $T_{max}$ ±RSD (h) | $t_{1/2}$ ±RSD (h) |
|---|---|---|---|---|
| Anagrelide | 19.46 ± 67% | 6.22 ± 62% | 2.0 ± 68% | 1.7± 47% |
| 3-HA (BCH24426) | 44.06 ± 40% | 8.74 ± 37% | 2.3 ± 54% | 3.87± 44% |
| RL603 | 32.07± 102% | 3.20 ± 71.4% | 3.8 ± 77.9% | 8.69 ± 55.3% |

**Attachment G Figure 2: Mean plasma concentrations of anagrelide, 3-hydroxy anagrelide and RL603 in ET patients at steady state**




### *1.3 Comparison of patient and volunteer PK data*

The pharmacokinetic parameters observed in ET patients do, in several respects, show similarity with those data generated in volunteers. Absorption was comparably rapid with a $T_{max}$ at 2h versus 1.3h seen in volunteers. Peak plasma concentrations of anagrelide and its subsequent half- life of elimination were reasonably similar being 6.22 and 4.99 ng/ml and 1.5 and 1.7h in patients and volunteers respectively.

However the most notable difference was seen in the pharmacokinetics of the active metabolite. In the patient group the mean $C_{max}$ value was 8.74 ± 37% ng/ml compared to 5.47 ± 57% ng/ml in volunteers but the most profound difference was in the exposure being 44.06 ± 40.5% ng·h/mL in patients compared to 18.0 ± 35.6% ng·h/mL in volunteers. Interestingly the half-life of elimination of 3-HA

was longer in patients than in volunteers being 3.9h compared to 2.5h respectively which could account for this greater exposure. This difference is unlikely to be simply due to age since in this respect the subjects were quite similar, with the volunteers ages ranging from 21-70yrs, mean 52 compared to the patients, 16 - 86yrs, mean 63.

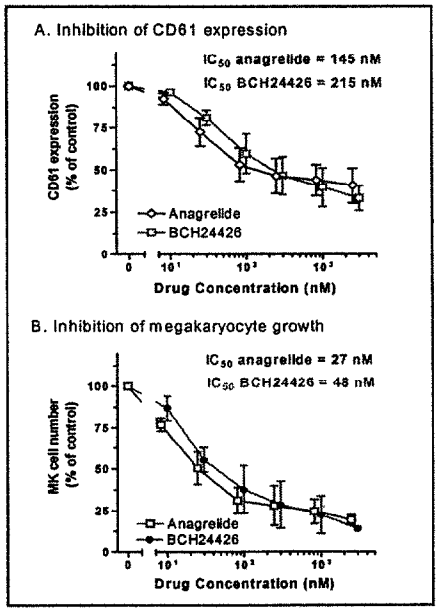
## 3. Pharmacology of anagrelide and its metabolites

### *3.1 Primary pharmacology - <u>in vitro</u> screening for platelet lowering potential*

The effects of anagrelide and its metabolites on the differentiation of human $CD34^+$ stem cells to megakaryocytes was assessed using a well established model of megakaryocytopoeisis (Cohen-Solal 1997, Cramer 1997). RL603 was found to be inactive in this model (Erusalimsky, Hong & Franklin 2002) despite earlier reports to the contrary (Lane et al 2001). Consequently it is now considered unlikely that this compound (RL603) contributes to the drug products therapeutic effects.

By contrast, 3-hydroxy anagrelide was found to have a comparable $IC_{50}$ value to the parent drug (anagrelide) in affecting the extent of megakaryocyte growth and differentiation ( see Figure 3 below). The most marked effect, like anagrelide, was on cell growth. The mean results from several studies showed anagrelide and 3-hydroxy anagrelide to be comparable in their potency to inhibit megakaryocyte growth and differentiation (and ultimately therefore blood platelet formation), having mean $IC_{50}$ values for the former process of 27nM and 48nM respectively.

**Attachment G Figure 3: Effects of anagrelide and its metabolite BCH24426 (3-HA) on megakaryocytopoeisis**



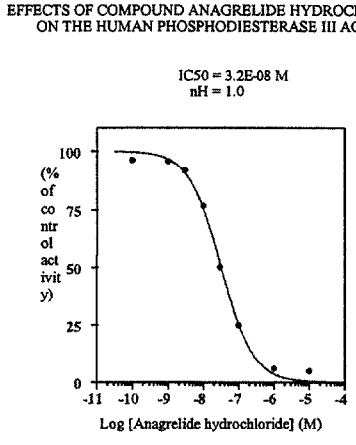
On this basis it is probable that 3-hydroxy anagrelide would make a substantial contribution to the platelet lowering effects of anagrelide especially in view of the clinically observed greater exposure (> 2 fold) to this metabolite than to anagrelide in ET/MPD patients. Furthermore while it is difficult to confidently extrapolate *in vitro* data to the *in vivo* clinical setting due to factors such as plasma protein binding* it is interesting to note that the $IC_{50}$ values for anagrelide and 3-hydroxy anagrelide - equivalent to 7 and 13ng/ml respectively - are of the same order the observed maximum plasma concentrations of anagrelide and its active metabolite seen in patients treated with the drug product.

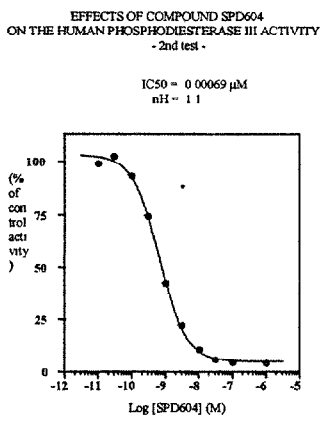
*The *in vitro* assay examining the effects of compounds on megakaryocytopoeisis contained 10% umbilical cord blood plasma which would effectively account for any inherent differences in plasma protein binding.

### *3.2 Secondary pharmacology - consequences of inhibition of PDEIII*

As anagrelide was already known to inhibit PDEIII, the activity of the metabolites was examined using enzyme derived from human platelets. The active metabolite, 3-hydroxy anagrelide was found to be nearly 40 times more potent than anagrelide itself having an $IC_{50}$ value of ~0.7nM (see Figure 4) This was verified in a second study resulting in average $IC_{50}$ of 0.9nM. By contrast RL603 was essentially inactive. Again while acknowledging the potential pitfalls in *in vitro* - *in vivo* extrapolations it is interesting to note that the clinically observed $C_{max}$(~9ng/ml) for BCH24426 was approximately 33 fold higher than its *in vitro* $IC_{50}$ for inhibition of PDEIII. By comparison, the $C_{max}$ concentrations for anagrelide would amount to only about three quarters the $IC_{50}$ for its inhibition of PDEIII. The other metabolite of anagrelide, RL603, was an extremely weak inhibitor of PDEIII with an $IC_{50}$ as high as 40,000nM.

**Attachment G Figure 4: Effects of anagrelide and 3-HA (SPD604) on human PDEIII**



The expected *in vivo* cardiovascular consequences of the PDEIII inhibition such as positive inotropic, chronotropic and vasodilatory activity have been reported previously for anagrelide itself. Recent studies have focussed on examining 3-hydroxy anagrelide in the anaesthetised dog model comparing it to the reference PDEIII inhibitor milrinone. These studies showed 3-HA to have a qualitatively similar cardiovascular profile to milrinone although it was at least 10-20 times more potent. In view of the very much greater potency of 3-hydroxy anagrelide to anagelide in this respect - and the greater plasma exposure seen clinically (metabolite to drug ratio being 2.3:1 - it would seem likely that this metabolite is the major contributor to the observed cardiovascular side effects of the drug product seen in man.

The other anticipated effect of inhibition of PDEIII namely platelet anti-aggregatory activity, was demonstrated for both anagrelide and 3-hydroxy anagrelide (although not evident with RL603). Anagrelide inhibited collagen induced platelet aggregation in human platelet enriched plasma but this effect was only substantial at relatively high concentrations of ~250ng/ml (much higher than would be observed clinically where the usual $C_{max}$ is ~6ng/ml). By contrast 3-HA was much more potent having an $IC_{50}$ of 0.053uM (14ng/ml) within the range encountered clinically (4-16ng/ml after a 1mg dose). RL603 had no effect on aggregation at concentrations up to 1000ng/ml, well above those encountered clinically.

## 4. Clinical pharmacokinetic – pharmacodynamic correlations.

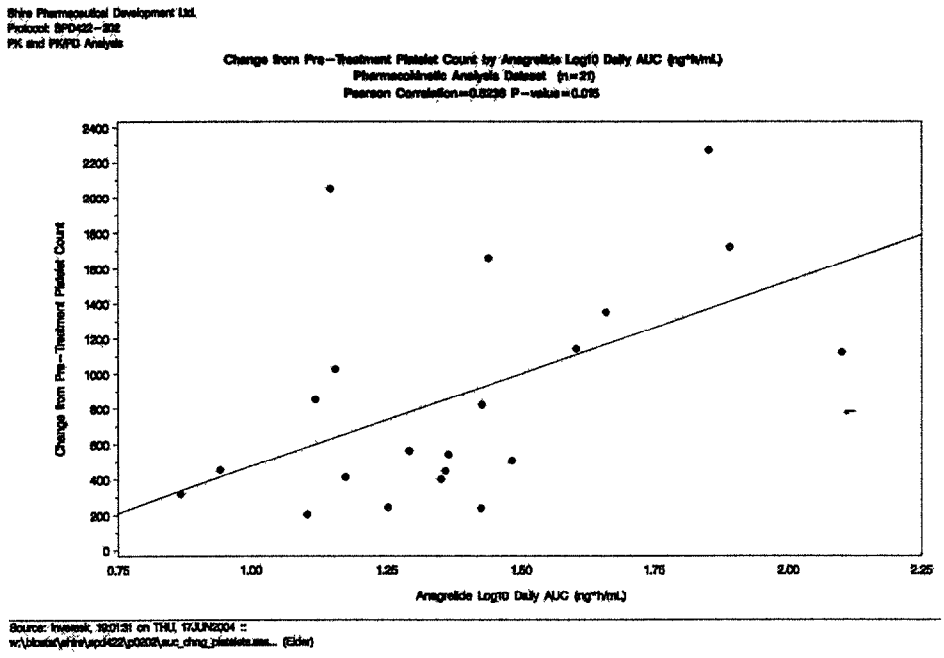
The possibility of a correlation existing between plasma anagrelide and/or active metabolite concentrations and the drug's clinical effects was explored in a group of ET patients being treated with anagrelide. Since the thrombocytopenic effects of anagrelide are only seen following extended multiple dosing, the possible relationship between *chronic* exposure to anagrelide and metabolite (AUC) and change in platelet count (over original baseline values) was examined in a log-linear model. Since the cardiovascular side effects of the drug product such as tachycardia occur acutely this was thought to be more likely related to $C_{max}$ than total exposure. Hence an exploration of the relationship between these levels and heart rate was made for both anagrelide and active metabolite again using a log-linear model.

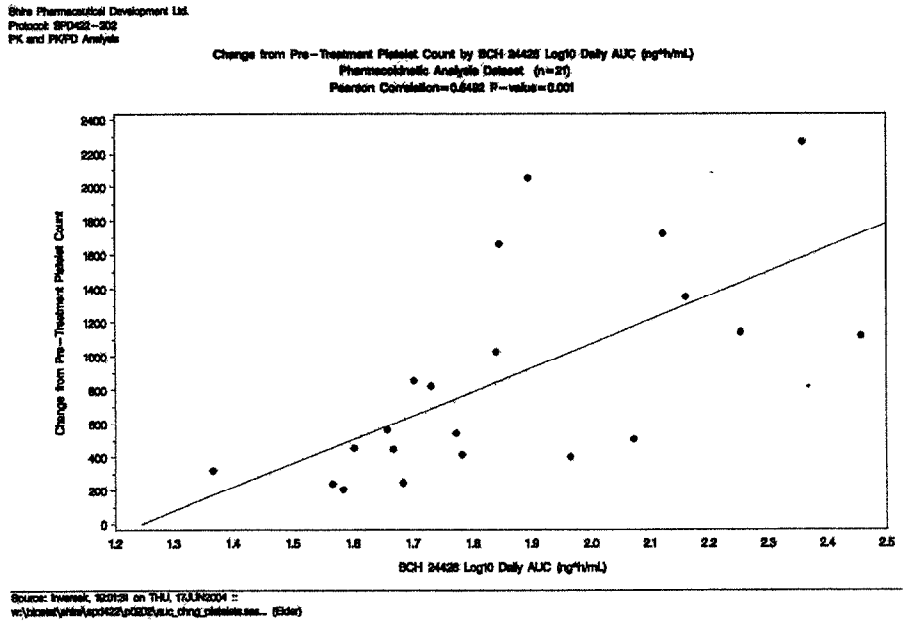
The results of the correlation analysis with platelet count are presented in Table 4 and Figure 5 below. The results show a stronger correlation between log plasma concentrations of the metabolite and changes in platelet count than for anagrelide itself.

**Attachment G Table 4: Correlation coefficients for log AUC at steady state and change in platelet count in ET patients**

| Compound & correlation analysis | N | Pearson correlation coefficient | Probability value |
|---|---|---|---|
| Log anagrelide AUC/ platelet count | 21 | 0.5236 | 0.015 |
| Log BCH24426 AUC/ platelet count | 21 | 0.6492 | 0.001 |

**Attachment G Figure 5: Relationship between log anagrelide or 3-hydroxy anagrelide (BCH24426) exposure (AUC) and change in platelet count at steady state in ET patients**



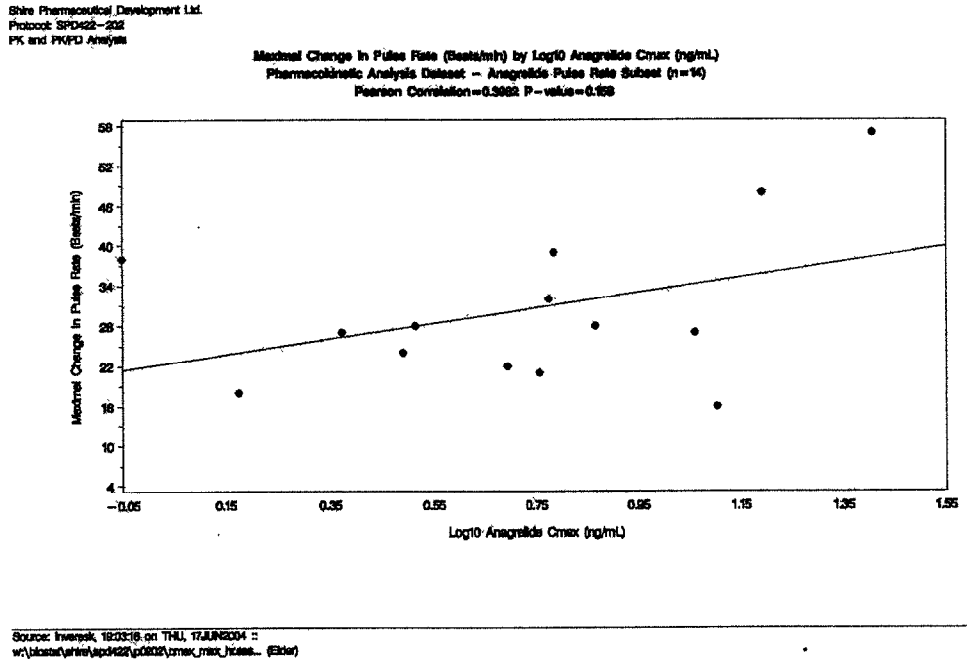



Examination of the correlation with pulse rate showed no correlation between anagrelide $C_{max}$ and pulse rate but an excellent correlation for the active metabolite and pulse rate as shown in Table 5 and Figures 6a and b.

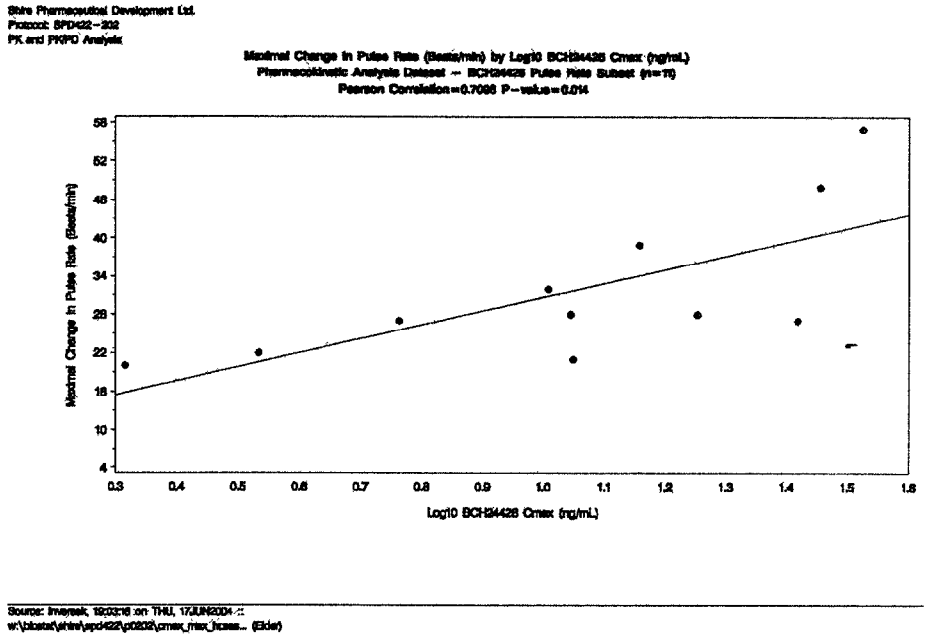
**Attachment G Table 5: Correlation coefficients for log Cmax and pulse rate**

| Compound & correlation analysis | N | Pearson correlation coefficient | Probability value |
|---|---|---|---|
| Log Anagrelide $C_{max}$/ pulse rate | 14 | 0.3962 | 0.158 |
| Log BCH24426 $C_{max}$/pulse rate | 11 | 0.7096 | 0.014 |

**Attachment G Figure 6a: Relationship between log of anagrelide exposure (AUC) and change in pulse rate at steady state in ET patients**



**Attachment G Figure 6b: Relationship between log 3-hydroxy anagrelide (BCH24426) exposure (AUC) and change in pulse rate at steady state in ET patients**




These correlations are entirely consistent with the proposed relative importance of anagrelide and 3HA in the drug products pharmacological activity.

## 5. Overall conclusions from the clinical pharmacokinetic and pharmacology studies on the active metabolite's (3-hydroxy anagrelide) role in the drug product activity.

A clinical study in patients has shown 3-hydroxy anagrelide to be the major entity circulating in the plasma of subjects with essential thrombocythemia or other MPDs. *In vitro* studies on the effects of this metabolite on the process of megakaryocytopoeisis suggest that it will have comparable platelet lowering activity to that seen for anagrelide since the compounds appear to be of a similar *in vitro* potency. By contrast, the other metabolite of anagrelide, RL603, is inactive. The predictability of this extrapolation from an *in vitro* screen into *in vivo* platelet lowering activity is supported by data on other compounds. For example hydroxyurea has been shown to inhibit megakaryocytopoeisis in this cell culture model, effects that are known to translate into clinical activity in terms of platelet lowering. In attempting to

transpose this *in vitro* activity into the *in vivo* setting differential factors affecting local availability of the compounds at the site of action (bone marrow) could influence the observed clinical activity e.g. plasma protein binding. However the relative activity of anagrelide and its active metabolite, 3-hydroxy anagrelide, is likely to be maintained since the assay for megakaryocytopoeisis included 10% umbilical cord blood plasma. Thus it might be reasonably assumed that the relative activity *in vitro* may be reflected by comparability *in vivo* . Since the active metabolite accounts for approximately 70% of the therapeutically active entities circulating in blood then given this comparability of potency with anagrelide it is likely to account for most of the platelet lowering activity of the drug product Agrylin. That 3-hydroxy probably plays such a major role in the platelet reducing properties of the drug product is reflected by the stronger PK-PD correlation for this compound rather than for anagrelide itself.

With respect to the contribution the metabolites make to the observed cardiovascular side effects of anagrelide (tachycardia and palpitations), RL603 being essentially inactive as a PDEIII inhibitor is unlikely to play any role but 3-hydroxy anagrelide which is 40 times more potent than anagrelide itself as an inhibitor of PDEIII will be the dominant contributor. Once again however the question might arise as to the predictability of the *in vivo* cardiovascular (CVS) effects from the *in vitro* inhibition of PDEIII. The consequences of this inhibition would be to increase levels of cyclic AMP, a second messenger, resulting in increased myocardial contractility and vasodilation in the venous and arterial circulation. There are many examples of drugs where this *in vitro* inhibition of PDEIII has been shown to predictably translate into CVS effects in man e.g. milrinone, amrinone enoximone, olprinone etc (Movsesian 2003). Once again however differential anagrelide and metabolite availability at the site of action - here the myocardium - could be determined by factors such as differential non-specific tissue and/or plasma protein binding. However the magnitude of the difference in the respective potencies to inhibit PDEIII are so large that such differences would most likely to be preserved *in vivo*. Finally investigation of the PK-PD between pulse rate and anagrelide or 3-HA $C_{max}$ showed evidence only for such a relationship with the metabolite.

In summary the investigations carried out consistently indicate that the most appropriate barometer for assessing Agrylin's biological effects would be plasma levels of 3-hdyroxy anagrelide rather than those of the parent drug.

## 6. References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